



Plasmid Transfection

< [Transfection Applications](#)

Plasmid Transfection

- [Optimization of Plasmid DNA Transfection](#)

[Stable Transfection](#)

[Co-Transfection](#)

[Transient Transfection](#)

[In Vivo Transfection](#)

[mRNA Delivery Technology](#)

[RNAi and Non-coding RNA Research](#)

Plasmid DNA remains the most common vector for transfection. DNA plasmids containing recombinant genes and regulatory elements can be transfected into cells to study gene function and regulation, mutational analysis and biochemical characterization of gene products, and effects of gene expression on the health and life cycle of cells. In addition, plasmid transfection can be used in large-scale [production of proteins](#) for purification and downstream applications in biotechnology and biopharmaceuticals.

In contrast to [mRNA transfection](#), DNA plasmid transfection requires nuclear entry prior to gene expression (**Figure 1**).

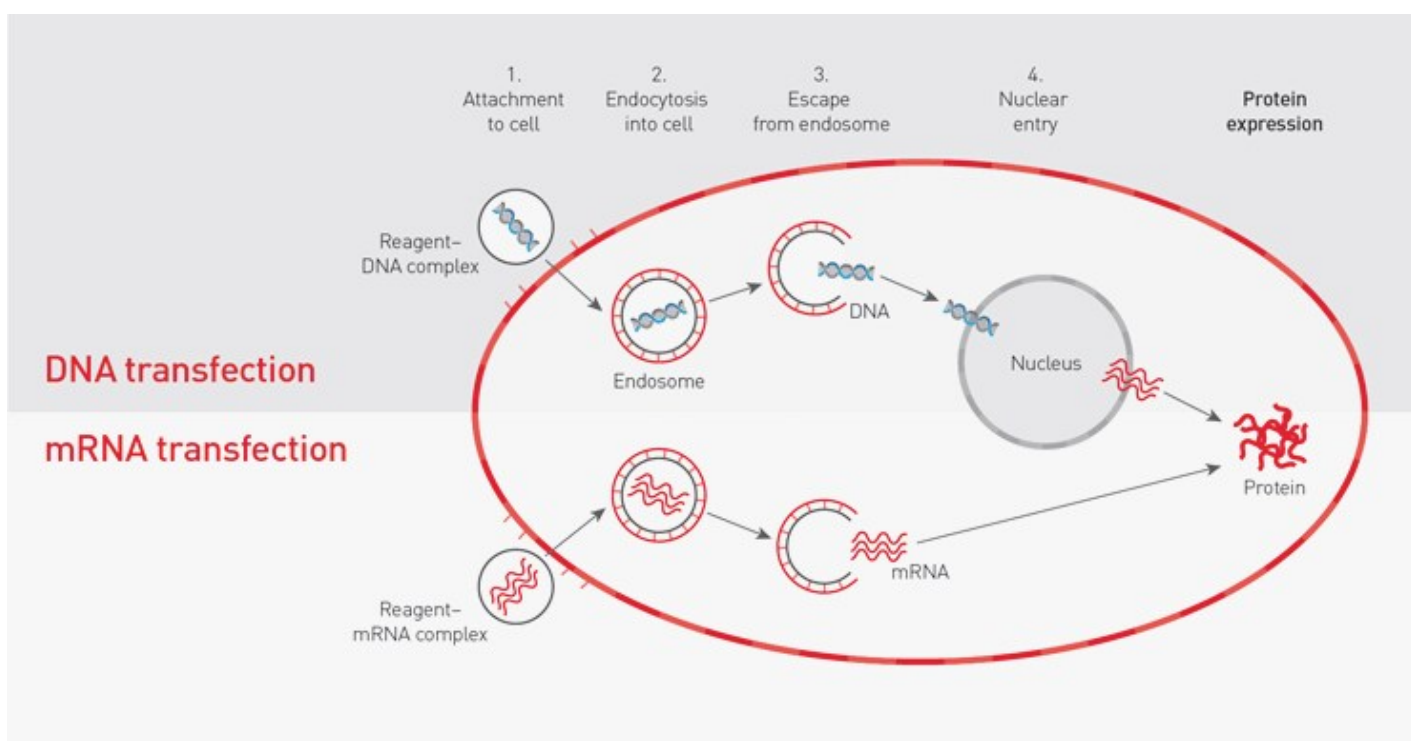


Figure 1. Differences between DNA and mRNA transfection. Unlike transfected mRNA, transfected DNA must enter the nucleus prior to being expressed.

Click image to enlarge

Guidelines and considerations for transfecting cells with plasmid

The topology (linear or supercoiled) and the size of the vector construct, the quality of the plasmid DNA, and the promoter choice are major factors that influence the efficiency of plasmid transfection.

[Learn more about optimizing plasmid DNA transfection](#) >

Effects of vector structure on plasmid transfection

When transfecting cells with plasmid, it is important to consider the topology of the DNA vector. Highly supercoiled, circular vectors are generally more efficient than linear DNA vectors for performing [transient transfection](#). This is most likely because circular DNA is not vulnerable to exonucleases, while linear DNA fragments are quickly degraded by these enzymes [1,2].

In addition, complexation patterns between [cationic lipid reagents](#) and circular or linear DNA vectors may vary. Specifically, atomic force microscopy analysis shows that circular DNA forms compact spherical or cylindrical condensates with cationic lipids whereas linear plasmids show extended pearl necklace-like structures. Although the cationic lipid-mediated transfection of the more compact circular plasmids is likely to go through endocytosis, the pathway of entry for extended linearized DNA structures might be different and less efficient [2].

Although circular DNA is preferred for transient transfection, linear DNA vectors are more efficient for performing [stable transfections](#). This is due to the enhanced ability of linear DNA to integrate into the host genome. Specifically, even though linear DNA is taken up by cells less efficiently, linear DNA with free ends is more recombinogenic and more likely to be integrated into the host chromosome to yield stable transformants.

Impact of vector size on plasmid transfection

In addition to vector topology, vector size can also impact plasmid DNA transfection. Despite similar uptake efficiencies in cationic lipid-mediated transfection, nuclear delivery of large plasmids is compromised compared with small plasmid molecules. This effect is observed using equivalent mass or molar concentrations of different-sized constructs, suggesting that nuclear delivery of plasmids may be limited by the rate of intracellular transit and that small plasmids evade degradation by rapid transit through the cytoplasm, rather than through the saturation of cellular defenses [1,3].

Quality of transfected plasmids

Purity and quality of the transfected plasmid DNA is also critical for a success. The best results are achieved with plasmid DNA of the highest purity that is free from phenol, sodium chloride, and endotoxins. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. Endotoxins, also known as lipopolysaccharides, are released during the lysis step of plasmid preparations and are often co-purified with plasmid DNA. Their presence sharply reduces transfection efficiency in primary and other sensitive cells.

Thermo Fisher Scientific offers a variety of kit options, including those for low endotoxin or endotoxin-free plasmid purification.

[Explore Invitrogen plasmid isolation kits](#) >

Although cesium chloride banding can also yield highly purified DNA, it is a labor intensive and time-consuming process. Excess vortexing of DNA-lipid complexes or DNA solutions may result in some shearing, especially with larger molecules, thereby reducing transfection efficiency. In addition, the concentration of EDTA in the diluted DNA should not exceed 0.3 mM.

Promoters for DNA plasmids

Promoter choice is dependent on the host cell line, the protein to be expressed, and the level of expression desired. Many researchers use the strong **CMV (cytomegalovirus) promoter** because it provides the highest expression activity in a broad range of cell types. Another strong promoter for high-level protein expression in mammalian cells is the **EF-1 α (human elongation factor-1 α)**. However, using too strong a promoter to drive the expression of a potentially toxic gene can cause problems in transfection of plasmid DNA. Therefore, for the potentially toxic gene products, use of weak promoters is recommended.

Stable transfection of toxic gene product plasmids

Toxic gene products can also be a problem for selection of stably transfected cells. For example, cells expressing a gene for antibiotic resistance can lose their growth advantage when such gene expression is detrimental to the health of the transfected cell. This can make it impossible to obtain stably transfected clones using a constitutive promoter. In such cases, an **inducible promoter** can be used to control the timing of gene expression, allowing for the selection of stable transfectants. Inducible promoters normally require the presence of an inducer molecule (e.g., a metal ion, metabolite, or hormone) to function. However, some inducible promoters function in the opposite manner, that is, gene expression is induced in the absence of a specific molecule.

Cell-type specific promoters, such as the polyhedrin promoter for insect cell expression, are also common. Literature searches are the best tool to determine which promoter will work best for your cell line or application.

Controls used for plasmid DNA transfection

Regardless of the transfection method used, it is important to perform control transfections to check for cell health, to determine whether the reported assay is working properly, and to establish any insert-related problems.

- To check for optimal cell growth conditions, include a negative control (no DNA, no transfection reagent).
- To establish that the reporter assay is working properly, include a positive control (parallel transfection with established transfection method).
- To determine whether there are insert-related problems, transfect a plasmid without the gene of interest.

Are you performing *in vitro* or *in vivo* transfection?

in vitro

in vivo

TransfectionSelect tool

Find products, citations, and protocols optimized for your transfection experiments. Input information on your experiment type, cell line, and payload to unlock solutions.

Visit [Transfection Basics](#) to learn more about performing transfection in your lab.

References

1. [McLenachan S, Sarsero JP, Ioannou PA. Flow-cytometric analysis of mouse embryonic stem cell lipofection using small and large DNA constructs. Genomics. 2007; 89\(6\): 708–720. ISSN 0888-7543.](#)
2. [Groll AV, Levin Y, Barbosa MC, Ravazzolo AP. \(2006\), Linear DNA Low Efficiency Transfection by Liposome Can Be Improved by the Use of Cationic Lipid as Charge Neutralizer. Biotechnol Progress, 22: 1220–1224.](#)
3. [Lukacs GL, Haggie P, Seksek O, Lechardeur D, Freedman N, Verkman AS. Size-dependent DNA mobility in cytoplasm and nucleus. J Biol Chem. 2000; 275\(3\):1625–1629. doi: 10.1074/jbc.275.3.1625. PMID: 10636854.](#)

For Research Use Only. Not for use in diagnostic procedures.

Ordering

[Order Status](#)
[Order Help](#)
[Quick Order](#)
[Supply Center](#)
[eProcurement](#)

Support

[Help and Support](#)
[Contact Us](#)
[Technical Support Centers](#)
[Documents and Certificates](#)
[Report a Site Issue](#)

Resources

[Learning Centers](#)
[Promotions](#)
[Events and Webinars](#)
[Social Media](#)

About Thermo Fisher

[About Us](#)
[Careers](#)
[Investors](#)
[News](#)
[Social Responsibility](#)
[Trademarks](#)

Our Portfolio

[Thermo Scientific](#)
[Applied Biosystems](#)
[Invitrogen](#)
[Gibco](#)
[Ion Torrent](#)
[Fisher Scientific](#)
[Patheon](#)
[PPD](#)